



United States
Department of
Agriculture

Agricultural
Research
Service

Northern Plains Area
Red River Valley Agricultural
Research Center
Biosciences Research
Laboratory

P.O. Box 5674
State University Station
Fargo, North Dakota
58105

November 3, 1988

SUBJECT: Summary of ARS Screwworm Attractants Workshop

TO: Recipients listed below

FROM: C.J. Whitten

Research Entomologist, Fargo, ND

Because an effective screwworm attractant is extremely important to the future success of the screwworm eradication program, not only as a surveillance tool but also as a supplement to reduce the overall screwworm population density, a workshop was held to address this specific subject. Participants included Agricultural Research Service research scientists and Animal and Plant Health Inspection Service screwworm program personnel.

The Workshop concluded that the first priority for future attractant research is to conduct a field comparison of known and potential screwworm attractants to determine the relative effectiveness of each. Depending on the results of this comparison, laboratory and field investigations will be focused on improvement of synthetic attractants, such as swormlure. For the long-term, research emphasis will be to critically define host-related attractant phenomena. An assessment and characterization of host wound ecology leading to the development of an artificial wound will be the emphasis of a medium-term commitment. Research attention will also be given to the role of plant attractants in adult screwworm population ecology.

Enclosed for your consideration, please find a summary report of the ARS Screwworm Attractants Workshop.

Recipients:

All participants

AD, Northern Plains Area, ARS (T.J. Army)

AD, Southern Plains Area, ARS (F.P. Horn)

AD, Pacific West Area, ARS (W.G. Chase)

AD, South Atlantic Area, ARS (E.L. Corley)

DA, National Program Staff, ARS (G.R. Evans)

ADA, Animal and Post Harvest Sciences, NPS, ARS (R.R. Oltjen)

ADA, Plant and Natural Resource Sciences, NPS, ARS (W. Klassen)

Associate Director, International Services, APHIS (J.W. Wyss)

Senior Staff Entomologist, International Services, APHIS (W. Sudlow)

Co-Director, Screwworm Eradication Program, APHIS (J.E. Novy)

Deputy Administrator, Veterinary Services, APHIS (L. King)

Director, Insect Attractants Laboratory, ARS (H. Oberlander)

ARS SCREWORM ATTRACTANTS WORKSHOP

SUMMARY REPORT

Fargo, ND, Oct. 4-5, 1988

1. Executive Summary:

A workshop to discuss the status, needs, and priorities of research on attractant systems for screwworms was held in Fargo, ND, Oct. 4-5, 1988. The major goal of the workshop was to identify the attractant research necessary to improve detection and monitoring of adult screwworm populations for eradication and research programs. Participants included 18 ARS and APHIS entomologists, chemists, or geneticists from the U.S., Mexico, and Costa Rica. The main topics were swormlure (the synthetic chemical attractant), wound attractants, artificial wounds, and plant attractants. The requirements for future activities, expressed in priority order, are: (1) a field comparison of known and potential attractants to determine the relative effectiveness of each in order to evaluate whether further research is appropriate, especially in existing systems; (2) long term studies to define host related attractant phenomena; (3) a medium term study of wound ecology; and (4) a continuation of deriving attractant information from population ecology studies in the field. The research is to be accomplished by scientists assigned to Mexico, Costa Rica, and/or Fargo screwworm research laboratories as determined by available resources.

2. Workshop Objectives:

1. To review the present status of screwworm attractants research,
2. To identify new areas of research which will produce technologies applicable to the screwworm eradication program,
3. To establish priorities and define requirements for the successful completion of this research.

3. Participants and Agenda: See Appendices I and II.

4. Results for Objective 1. Review of Present Status

Swormlure - Currently, a wind-oriented trap (WOT) baited with swormlure-4 (SL-4) is the standard screwworm trapping system used by the research and eradication programs to trap male and female screwworms. However, this system, which performed adequately in more arid environs, does not appear to be as effective in the more tropical areas. Swormlure is the primary attractant ingredient used in SWASS, deployed for screwworm population suppression at critical times. The evolution of SL-4 and results of unpublished research were discussed. SL-4 is the most effective swormlure formulation

developed and is 35-50% more effective than SL-2. Apparently, all 10 components of the SL-4 chemical mixture are necessary as omission of any one results in a 50% reduction in effectiveness. Attempts to incorporate SL-4 into slow-release formulations have not been successful, probably because of the caustic nature of the chemical components and the volume necessary. The attraction mechanism (plant or animal related) of swormlure is uncertain. CSIRO scientists in New Guinea are also using SL-4 but with a different trap design; their data indicate that the trapping system is less effective than wounded animals for collecting Chrysomya bezziana, the Old World screwworm. Neither ARS nor CSIRO have current efforts to improve swormlure.

Wound Attractants - In the field, female screwworms respond to animal wounds for oviposition and feeding. In the lab, gravid or vitellogenic non-fed (protein source) females have demonstrated a response to wound fluids, air passed over active wounds, navel cord preparations, culture preparations of the bacterium Providencia rettgeri, and dried bovine blood. No olfactory response was reported for fresh wounds or whole blood. The results suggest an involvement of bacterially generated products. The least variable and most linear dosage related response was elicited with the dried blood. For this reason and due to the ample supply of the product, subsequent research has utilized the dried blood as the standard for laboratory studies. Basically total activity of the blood can be recovered in steam distillate preparations and after HPLC fractionation of the distillate, 4 active HPLC fractions appear to be synergistic in combinations. Preliminary tests with dried blood preparations have not demonstrated attraction in the field. The role of a host-attractant interaction has not been studied.

Artificial Wounds - Collections of screwworm eggs are needed to determine the efficiency of the sterile fly release program as it proceeds into new areas of Central America. The development of a system to duplicate animal wounds is becoming more imperative not only because of the high cost of maintaining wounded animals but also because of recent legislation and public concern about using animals for research purposes. Initial attempts to develop a workable model of an artificial wound has been hindered by the lack of information about ovipositional and feeding stimuli. Some screwworm eggs have been collected by using meats placed inside a heated unit. However, oviposition by other blow fly species has reduced the efficiency of these devices. When information becomes available on the factors that influence egg deposition, more serious attempts to develop an artificial device to collect eggs will be pursued. Presently, a trap designed to be attached over the wound of the sentinel animal is being used to collect flies responding to the wound.

Plant Attractants - Both sexes of screwworm flies have been observed visiting flowers of plants but the actual mechanism of attraction is not known. The flies may visit flowers in response to volatiles and/or color or shape of the blossom. Screwworms have been found primarily on flowers in which the fly could insert its mouthparts. Extrafloral sources were no more attractive than flowers in outdoor cage studies. Certain Acacia species are among the attractive plants and the volatiles (114 total) associated with blossoms of 3 species have been identified. However, attempts to elicit a response to the blossoms (after being collected fresh and stored frozen) in an olfactometer situation have not been successful. It is not known what effect the sampling of blossoms has on attractiveness in laboratory tests. Presently, pollen collected from screwworms in Central America is being identified to establish the primary plants visited by screwworms in a tropical environment.

Other Attractants - The response to any of the above attractants may well be explained by a feeding response and, if physiological conditions for the screwworm female and the nutrient source (ie. the wound) are correct, oviposition may occur. In Costa Rica, a screwworm adult feeding station containing rotting liver has attracted significantly more screwworms than a wounded animal.

5. Results for Objective 2. Identification of Needed Research

A. Swormlure:

1. Analyze volatiles of SL-4 with time to improve chemical formulation.
2. Test grouping of chemicals to improve effectiveness.
3. Improve trap design and formulation for slow release.

B. Wound Attractants

1. Is swormlure as effective as wounded animals, liver feeding stations, or dried blood?
2. Define host related phenomena
 - a. Attraction
 - 1) Develop bioassay.
 - 2) Sampling and collecting potential volatiles.
 - 3) Isolation and identification of chemicals responsible for response.
 - 4) Synthesis and formulation of chemicals with attractant properties.
 - 5) Field evaluation of attractant chemicals.
 - b. Stimuli (whole animal concept)
 - 1) Biological
 - 2) Physical
 - 3) Chemical

3. Wound Ecology

- a. Larval effects
- b. Bacterial effects
- c. Host physiology

C. Artificial Wounds

- 1. Ovipositional and feeding stimuli
 - a. Chemical aspects (to include fresh whole blood).
 - b. Physical aspects
 - c. Behavioral aspects (physiological controls).
- 2. Design concepts (mechanical aspects).

D. Plant Attractants

- 1. Determine (identify) plants most frequently visited.
- 2. Identify stimuli responsible for response (ie. color, shape, odor, etc.).
- 3. Apply procedure used for wound attractant attraction components as applicable leading to field evaluation.

6. Results for Objective 3. Priorities and Interaction.

- 1. Test effectiveness of SL-4 in comparison with wounded animals, liver feeding stations, and dried blood in the 3 different ecological zones of Central America. (B.1 above) To be done in Costa Rica through cooperative effort of ARS and APHIS locations in Mexico.
- 2. Define host related phenomena. (B.2) Long term project with probable interaction of Fargo (such as the development of a wind-tunnel study) and Costa Rica (biological and physiological aspects) laboratories with cooperation of Mexico laboratory.
- 3. Wound ecology. (B.3) Medium term commitment with primary work proposed for Fargo (with the possibility of the assistance of a microbiologist) and the cooperation of the Mexico laboratory.
- 4. Improvement of swormlure. (A.1-3) Dependent on results of Priority 1 (ie. if swormlure shows potential).
- 5. Artificial wounds. (C.1-2) Low input - sideline activity with field studies in Mexico and Costa Rica.
- 6. Plant attractants. (D.1-3) Adult screwworm-plant relationships are important in population ecology studies in Central America; significant results could increase priority for further attractancy studies.

APPENDIX I

LIST OF WORKSHOP PARTICIPANTS

Adams, T.S.	Research Entomologist	ARS, Fargo, ND
Ashley, T.	Entomologist	APHIS, Tuxtla-Gtz., Mexico
Bram, R.A.	National Program Leader	ARS, Beltsville, MD
Flath, R.A.	Research Chemist	ARS, Albany, CA
Friese, D.D.	Research Entomologist	ARS, Tuxtla-Gtz., Mexico
Gassner, G.G.	Research Geneticist, RL	ARS, Fargo, ND
Gersabeck, E.	Entomologist	APHIS, Tuxtla-Gtz., Mexico
Hammack, L.	Research Entomologist	ARS, Fargo, ND
Hofmann, H.C.	Staff Entomologist	APHIS, Mexico City, Mexico
Mackley, J.W.	Entomologist	APHIS, Tuxtla-Gtz, Mexico
Mangan, R.L.	Research Entomologist, RL	ARS, Tuxtla-Gtz., Mexico
McDonald, I.C.	Research Geneticist	ARS, Fargo, ND
Parker, F.D.	Research Entomologist	ARS, San Jose, Costa Rica
Peterson, R.D.	Research Entomologist	ARS, Fargo, ND
Pomonis, J.G.	Research Chemist	ARS, Fargo, ND
Schmidt, C.H.	Research Entomologist	ARS (Ret), Fargo, ND
Tumlinson, J.H.	Research Chemist	ARS, Gainesville, FL
Whitten, C.J.	Research Entomologist	ARS, Fargo, ND

APPENDIX II

ARS WORKSHOP ON ATTRACTANTS FOR SCREWORMS

O'Leary Room, Kelly Inn
Fargo, ND
October, 4-5, 1988

AGENDA

OCTOBER 4 - C.J. Whitten, Chairperson

8:30	Opening and Introductions	
	<u>Status Reports</u>	
8:45	Status Report on Swormlure	Mackley/Whitten
9:15	Discussion	Participants
9:30	Status Report on Wound Attractants	Hammack/Pomonis/Flath
10:00	Discussion	Participants
10:15	BREAK	
10:30	Status Report on Artificial Wounds	Mangan/Parker
11:00	Discussion	Participants
11:15	Status Report on Plant Attractants	Peterson/Parker
11:45	Discussion	Mangan Participants
12:00	LUNCH - Kelly Inn	
1:30	Status Report on Other Attractants (Feeding, Oviposition, etc.)	Peterson/Hammack
2:00	Discussion	Participants
	<u>Identification of Needed Research</u>	
2:15	Swormlure	Participants
2:45	Wound Attractants	Participants
3:15	BREAK	
3:30	Artificial Wounds	Participants
4:00	Plant Attractants	Participants
4:30	Other Attractants	Participants
	DINNER - Trader & Trapper, Moorhead	Participants

October 5 - Ralph Bram, Chairperson

Priorities and Resources

8:30	Establish Research Priorities	Participants
9:30	Determination of Resources	Participants
10:00	BREAK	
10:15	Areas of Cooperation and Interaction	Bram
10:45	Workshop Summary	Bram
	LUNCH	

Remainder of available time for participants tour of screwworm lab,
Biosciences Research Lab and/or visitation with scientists.



United States
Department of
Agriculture

Agricultural
Research
Service

North Central Region
Metabolism and
Radiation Research
Laboratory

P.O. Box 5674
State University Station
Fargo, North Dakota
58105

January 22, 1989

SUBJECT: Plans for Research in Costa Rica during February 1989

TO: R.D. Peterson, Research Entomologist
Leslie Hammack, Research Entomologist

FROM: C.J. Whitten, Lead Scientist *CJ Whitten*

The scientists in San Jose, Costa Rica, have requested that you send a brief outline which includes equipment and support needs anticipated for the work planned during your visit to Costa Rica in February. Such information will facilitate better scheduling of equipment and technician support as well as U.S. embassy notification for field work.

In addition, I am asking you to participate as a member of a team, consisting of yourselves and Frank Parker, to prepare recommendations and preliminary plans for the completion of the 1st priority established by the Attractants Workshop in October, 1988. (Please recall the objective of the research is to test the effectiveness of swormlure-4 in comparison with wounded animals, liver feeding stations, and dried blood in the different ecological zones of Central America.) The preliminary plan should include a proposed experimental design with time and support requirements necessary for this important phase of attractants research.

cc: R.A. Bram, Beltsville
J.B. Welch, Costa Rica
F.D. Parker, Costa Rica
I.C. McDonald, Fargo



United States
Department of
Agriculture

Agricultural
Research
Service

Northern Plains Area
Red River Valley Agricultural
Research Center
Biosciences Research
Laboratory

P.O. Box 5674
State University Station
Fargo, North Dakota
58105

14 March 1989

SUBJECT: Draft Proposal for Attractants Field Test

TO: J. B. Welch, Location Leader
F. D. Parker, Research Entomologist
D. Friese, Research Entomologist ✓

FROM: R. D. Peterson, Res. Entomologist

Enclosed find the first draft of the proposal for an attractants field test in Costa Rica. This draft is a result of our meetings in San Jose on 16-17 February 1989.

Please send me your suggestions and comments and I will make any changes before our final recommendations are circulated.

Pete

Richard D. Peterson II
ARS-USDA-Biosciences Research Laboratory
P. O. Box 5674
Fargo, ND 58105

Pete,

Overall I think this is a well presented report which covers thoroughly what was discussed at the meeting. I have included a few comments which you may or may not include in the final draft. I am pleased I took the others with me as well.

Van

The first priority of the Screwworm Attractants Workshop held 4-5 October 1988 in Fargo, ND was to conduct a field test in Costa Rica to measure the effectiveness of swormlure in comparison with wounded animals, liver feeding stations, and dried blood solution. All but the dried blood are currently being tested in Costa Rica or have been studied previously from Texas to Belize. Generally, liver has caught the most screwworms, followed by wounds and then swormlure. (TABLE 1) The validity of comparisons among studies is often compromised by methodological differences, and low catches can be a result of a poor attractant, low screwworm populations, trap efficiency, or poor trap sites.

The new field test would compare relative attractiveness based on the number of flies captured while visiting the bait. The attractants could be tested concurrently using any of the following methods:

- A. Traditional. Using methods described in the literature.
- B. Standard. Using methods currently in use.
- C. Quantitative. Materials placed on the ground with uniform exposed surface area.

What follows is a comparison of each method along with the advantages, disadvantages, and limitations of each. The test constraints are then discussed with general comments on feasibility in the Costa Rican context. This is followed by a proposal for a field test which we feel will address the question of attractiveness.

DRAFT

A. Traditional Method. Using methods described in the literature.

1. Infested animal wound. Egg masses collected twice daily.

Advantages:

- a. Field standards as used in strain evaluations, etc.
- b. A reference point for comparison to past results.

Disadvantages:

- a. Wounded animals can only be used in restricted areas.
- b. Cost of animal transport and maintenance.
- c. Most screwworms visiting wounds do not oviposit.

2. Dried blood solution in a wind oriented trap (WOT) or Bishopp trap.

Advantage: None

Disadvantages:

- a. No field data on suitability for use in either trap.
- b. Unknown chemical composition over time.
- c. Frequent theft of WOTs in all but select areas.

3. Swormlure-4 used in a WOT.

Advantages:

- a. Field standard as used in strain evaluations, etc.
- b. Known chemical composition.
- c. A reference point for comparison with past results.

Disadvantages:

- a. Unknown whether Costa Rica government would approve SL-4 use in more than a small scale field test.
- b. Frequent theft of WOTs in all but very selected areas.
- c. Handling of toxic material.

4. Liver used in a Bishopp Trap.

Advantage: Standard trapping system before the WOT.

Disadvantages:

- a. Traps large numbers on non-target species.
- b. Identification is labor intensive.

The limitations of the Traditional Method are as follows:

1. Presentation of attractant (WOT, animal, Bishopp) is not uniform.
2. Wounded animal will yield masses, not numbers of flies visiting the wound.
3. Replications of the four presentations would be different, ie. could not have as many wounded animals as WOT traps.
4. Trap efficiency and type of attractant cues are different.
5. The spacing between replicates and treatments would require a large area.
6. A study area of sufficient size does not at present exist.

The trap developed by Rubink to capture flies visiting the wound has not been described in the literature and therefore was not considered.

DRAFT

B. Standard Method. Using methods currently in use.

1. Infested animal wound. Flies collected on the bait by a technician.

Advantages: Technician collects all flies as they visit the wound.

Disadvantages:

- a. Wounded animals can only be used in restricted areas.
- b. Cost of animal transport and maintenance.
- c. Labor intensive.

Since dried blood has been tested in the field before I don't think we should have the presentation do the ground. Perhaps you could use 2a. Dried blood solution on the ground. 2b Dried blood solution in a WOT and list advantages & disadvantages for both

2. Dried blood solution on the ground. Flies collected on the bait by a technician.

Advantages: Technician collects all flies as they visit the bait.

Disadvantages:

- a. Periodic additions of dried blood solution
- b. Labor intensive.

3. Swormlure-4 used in a WOT.

Advantages:

- a. Field standard as used in strain evaluations, etc.
- b. Known chemical composition.
- c. A reference point for comparison with past results.

Advantages: More replicates could be run. Less labor intensive. Disadvantages: Frequent theft of WOTs in all but very selected areas.

Disadvantages:

- a. Unknown whether Costa Rica government would approve SL-4 use in more than a small scale field test.
- b. Frequent theft of WOTs in all but very selected areas.
- c. Handling of toxic material.

4. Liver on the ground. Flies collected on the bait by a technician.

Advantages:

- a. Technician collects all flies as they visit the liver.
- b. Presentation as used in ongoing population studies.

Disadvantages:

- a. Periodic turning of the liver and additions of water.
- b. Labor intensive.

The limitations of the Standard Method are as follows:

1. Replication would require more than doubling present manpower.
2. Presentation of attractant (WOT, animal, ground) is not uniform.
3. Replications of the four presentations would be different, ie. could not have as many wounded animals as WOT traps.
4. Trap efficiency and type of attractant cues are different.
5. The spacing between replicates and treatments would require a large area.
6. A study area of sufficient size does not at present exist.

Any merit the approach has lies in that 3 out of 4 of the test materials include a technician to collect flies as they visit the bait.

DRAFT

C. Quantitative Method. Test material placed on the ground with uniform exposed surface area.

1. Wound fluid. Soaked in cotton in uniform sized container.

Advantages: Presented in a standard context with the other three.

Disadvantages:

- a. Quantity of wound fluid would be prohibitive.
- b. Collection, storage, and transport of fluid to the field.
- c. Maintenance of an animal source for the fluid.
- d. Periodic additions of wound fluid.
- e. Labor intensive.

2. Dried blood solution. Soaked in cotton in uniform sized container.

Advantages: Presented in a standard context with the other three.

Disadvantages:

- a. Periodic additions of dried blood solution.
- b. Labor intensive.

3. Swormlure-4. Soaked in cotton in uniform sized container.

Advantages:

- a. Presented in a standard context with the other three.
- b. Known chemical composition.

Disadvantages:

- a. Periodic additions of SL-4.
- b. Labor intensive.
- c. Unknown whether rodenticide would approve ...
- d. Handling of toxic material

4. Liver. Cut to fit uniform sized container.

Advantages: Presented in a standard context with the other three.

Disadvantages:

- a. Periodic turning of the liver and additions of water.
- b. Labor intensive.

The limitations of the Quantitative Method are as follows:

1. Replication would require more than doubling present manpower.
2. The spacing between replicates and treatments would require a large area.
3. A study area of sufficient size does not at present exist.

The method has merit in that all 4 materials are exposed in the same manner.

DRAFT

GENERAL COMMENTS

Over the years, the ARS staff in Costa Rica has determined in which areas field research can be productively carried out. Theft of traps and animals has been the limiting factor in selecting study areas. As a deterrent to this problem, it has been suggested that ARS hire guards to patrol trap lines and penned animals. Only one study area, the experiment station near Canas (tropical dry forest), has proved satisfactory. (See MAP) The area is relatively small when compared to the area needed to test the four substances concurrently. There is the possibility that limited studies can also be conducted near La Selva (tropical wet forest), however, introduction of animals into the area is prohibited.

A large scale field test will require the hiring and training of additional technicians and further strain the resources of the Costa Rica laboratory. There are presently 4-5 technicians available for field work, and any large scale test would require at least doubling this figure. Bringing in trained technicians from Mexico would stretch Tuxtla's ^{labor and} ~~capabilities~~ ^{activities} and test Costa Rican immigration law. It was the consensus of the team that a short term field trial would best address the question of comparative attractiveness.

It is recommended that swormlure (SL-4) not be used as the population indicator to which the other attractants are compared. Swormlure baited traps have generally caught fewer flies compared with liver or infested animals (TABLE 1). In addition, swormlure is toxic, requires special handling, and is expensive. It is recommended that liver be used as the

DRAFT

population indicator to which comparisons are made. The choice of liver is based on its low cost, ease of use, and its ability to attract substantial numbers of screwworms everywhere in Costa Rica where it has been tested (TABLE 1). The components for making swormlure are unavailable on the open market in Costa Rica. Procedures to order, ship and import the ingredients are currently being worked out. It is hoped the chemicals will be available in the near future. It is unclear whether Costa Rican government approval is necessary before swormlure can be put into the environment.

To compare infested animals with the other attractants presents major problems of interpretation. Studies in Costa Rica at Canas (tropical dry forest), San Carlos (tropical moist forest) and Tres Rios (tropical premontane moist forest) have shown that both liver and infested animals can be effective attractants. To compare them head-to-head, however, is like comparing apples and oranges. Factors such as wound age and size, animal warmth and movement, and extraneous odors unassociated with the wound make direct comparisons invalid.

The staff in Costa Rica currently has research in progress in the Canas study area and have made research plans to use the area well into 1990. Interrupting these studies before they are complete would be a disservice to the researchers involved. In fact, data collected since the workshop indicates that some of the comparative data on attractiveness is already available (TABLE 1).

DRAFT

PROPOSAL

It was the consensus of the team that the effectiveness of SL-4 could be evaluated without an expensive, large scale field test with which the screwworm program has become associated. We recommend that paired comparisons between liver and dried blood solution, and liver and swormlure be conducted during the wet and dry season in two ecological zones of Costa Rica. ^{paired comparisons between liver and swormlure have previously been done and, could - also - be used in (reducing)} This will best address the question of attractiveness, both from the standpoint of test design and efficient use of limited resources.

It is proposed that the initial studies be conducted at the experiment station near Canas (tropical dry forest), and depending on the results, the study would be repeated in a second ecological zone such as at a site in or near the La Selva field station (tropical wet forest). The difficulty in locating suitable study areas and the current restriction on using live animals in La Selva have been previously noted. The initial field test would be conducted as soon as possible within the constraints of ongoing research.

The paired comparisons will run 10 consecutive days. Ten days is recommended to give each attractant equal advantage in attracting different segments of the population ie. newly emerged, feeding, mating or ovipositing. Based on past data, we expect the liver to be an excellent indicator of the presence of screwworms in the area.(TABLE 1) The attractants will be presented in uniform sized containers set on the ground. It is recommended that liver and infested animals not be compared because of the lack of standardized presentation.

DRAFT

There will be two sites for each paired comparison ie. one liver station vs one SL-4 station, etc. The treatments will be rotated daily to minimize any bias between sites. It will be necessary to make periodic additions of SL-4 and the dried blood solution to prevent the solutions from drying out. Additions of water and periodic turning of the liver will also be necessary to maintain its attractiveness. A technician will observe each treatment, capture the flies by net or vial upon visitation, hold those captured, and release them at the end of each day. This will minimize recaptures of released flies. Duration of the daily exposure will include the time when screwworms are known to be active.

Numbers of flies captured will be recorded by sex. The t-test for paired comparisons will be used to evaluate the treatments. The paired t-test is especially appropriate for this type of data to eliminate a source of extraneous variance (Steel and Torrie 1960).

It is hoped the evaluation can be conducted with minimal disruption to ongoing research activities. However, it is estimated that funds may need to be allocated to hire and train additional technicians to assist in the field. Additional expense would be incurred in increased per diem, vehicle operation and maintenance, and perhaps customs duty charges and security guards at the study sites. It should be pointed out that this field test will not answer the question of which is the "best" attractant. It will, however, provide information on the effectiveness of swormlure in selected areas of Costa Rica and on the advisability of continuing chemical isolation of the dried blood attractant.

DRAFT

REFERENCES

- Brenner, R. J. 1984. Dispersal, mating, and oviposition of the screwworm (Diptera: Calliphoridae) in southern Mexico. *Ann. Entomol. Soc. Amer.* 77:779-788.
- Brenner, R. J. 1985. Distribution of screwworms (Diptera: Calliphoridae) relative to land use and topography in the humid tropics of southern Mexico. *Ann. Entomol. Soc. Amer.* 78:433-439.
- Broce, A. B., J. L. Goodenough, and J. R. Coppedge. 1977. A wind oriented trap for screwworm flies. *J. Econ. Entomol.* 70(4):413-416.
- Coppedge, J. R. and J. W. Snow. 1977. Seasonal response of screwworm adults to two attractants in subtropical Texas. *Southwest Entomol.* 2(2):57-61.
- Coppedge, J. R., E. Ahrens, J. L. Goodenough, F. S. Guillot, and J. W. Snow. 1977. Field comparisons of liver and a new chemical mixture as attractants for the screwworm fly. *Environ. Entomol.* 6(1):66-68.
- Coppedge, J. R., J. L. Goodenough, A. B. Broce, F. H. Tannahill, J. W. Snow, M. M. Crystal, and H. D. V. Petersen. 1978. Evaluation of the screwworm adult suppression system (SWASS) on the island of Curacao. *J. Econ. Entomol.* 71(4):579-584.
- Jones, C. M., J. W. Snow, and M. A. Villasenor. 1976. Screwworm flies: Seasonal occurrence in central Tamaulipas, Mexico, 1973-74. *J. Econ. Entomol.* 69(6):761-762.
- Krafsur, E. S. and B. G. Hightower. 1979. Field tests of sterile screwworm flies, Cochliomyia hominivorax (Diptera: Calliphoridae), against natural populations in three coastal areas of Mexico. *J. Med. Entomol.* 16(1):33-42.
- Lindquist, A. W. 1938. A study of the incidence and habits of Cochliomyia americana by means of flytraps. *J. Kan. Entomol. Soc.* 11(3):97-104.
- Mackley, J. W. 1986. Incidence of the screwworm, Cochliomyia hominivorax, and the secondary screwworm, C. macellaria (Diptera: Calliphoridae), in the central highlands of Chiapas, Mexico. *J. Med. Entomol.* 23:76-82.
- Mackley, J. W. and H. E. Brown. 1984. Swormlure-4: A new formulation of swormlure-2 mixture as an attractant for adult screwworms, Cochliomyia hominivorax (Diptera: Calliphoridae). *J. Econ. Entomol.* 77:1264-1268.
- Spencer, J. P., C. J. Whitten, J. R. Coppedge, and J. W. Snow. 1980. Comparison of screwworm captures in liver- and swormlure-baited traps in a tropical area of southern Mexico. *Southwestern Entomol.* 5(3):175-178.

DRAFT

Spencer, J. P., J. W. Snow, J. R. Coppedge and C. J. Whitten. 1981. Seasonal occurrence of the primary and secondary screwworm (Diptera: Calliphoridae) in the Pacific coastal area of Chiapas, Mexico during 1978-1979. J. Med. Entomol. 18:240-243.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill: New York. 481p.

Thomas, D. B. and R. L. Mangan. Oviposition and wound visiting behavior of the screwworm fly, Cochliomyia hominivorax (Coquerel) (Diptera: Calliphoridae). Ann. Entomol. Soc. Amer. (in press).





United States
Department of
Agriculture

Agricultural
Research
Service

Northern Plains Area
Red River Valley Agricultural
Research Center
Biosciences Research
Laboratory

P.O. Box 5674
State University Station
Fargo, North Dakota
58105

November 3, 1988

SUBJECT: Summary of ARS Screwworm Attractants Workshop

TO: Recipients listed below

FROM: C.J. Whitten *CJ Whitten*
Research Entomologist, Fargo, ND

Because an effective screwworm attractant is extremely important to the future success of the screwworm eradication program, not only as a surveillance tool but also as a supplement to reduce the overall screwworm population density, a workshop was held to address this specific subject. Participants included Agricultural Research Service research scientists and Animal and Plant Health Inspection Service screwworm program personnel.

The Workshop concluded that the first priority for future attractant research is to conduct a field comparison of known and potential screwworm attractants to determine the relative effectiveness of each. Depending on the results of this comparison, laboratory and field investigations will be focused on improvement of synthetic attractants, such as swormlure. For the long-term, research emphasis will be to critically define host-related attractant phenomena. An assessment and characterization of host wound ecology leading to the development of an artificial wound will be the emphasis of a medium-term commitment. Research attention will also be given to the role of plant attractants in adult screwworm population ecology.

Enclosed for your consideration, please find a summary report of the ARS Screwworm Attractants Workshop.

Recipients:

All participants

AD, Northern Plains Area, ARS (T.J. Army)

AD, Southern Plains Area, ARS (F.P. Horn)

AD, Pacific West Area, ARS (W.G. Chase)

AD, South Atlantic Area, ARS (E.L. Corley)

DA, National Program Staff, ARS (G.R. Evans)

ADA, Animal and Post Harvest Sciences, NPS, ARS (R.R. Oltjen)

ADA, Plant and Natural Resource Sciences, NPS, ARS (W. Klassen)

Associate Director, International Services, APHIS (J.W. Wyss)

Senior Staff Entomologist, International Services, APHIS (W. Sudlow)

Co-Director, Screwworm Eradication Program, APHIS (J.E. Novy)

Deputy Administrator, Veterinary Services, APHIS (L. King)

Director, Insect Attractants Laboratory, ARS (H. Oberlander)



United States
Department of
Agriculture

Agricultural
Research
Service

Northern Plains Area
Red River Valley Agricultural
Research Center
Biosciences Research
Laboratory

P.O. Box 5674
State University Station
Fargo, North Dakota
58105

April 30, 1989

SUBJECT: Recommendations for Attractants Field Test

TO: C. J. Whitten, Workshop Moderator

FROM: R. D. Peterson II, Research Entomologist
ARS-Biosciences, Fargo, ND

In response to your memo of 22 January 1989, the following is submitted for review.

A meeting was held in San Jose, Costa Rica 16-17 February 1989 to discuss the test rational and possible designs of the field test to measure the effectiveness of swormlure (SL-4) in comparison with wounded animals, liver, and rehydrated blood. The San Jose meeting was in response to one of the recommendations of the Screwworm Attractants Workshop held 4-5 October 1988 in Fargo, ND.

The attached report represents the team members review of the Workshop recommendation and preliminary plans for an attractants field test.

cc: R. A. Bram
D. R. Nelson
Team Members ✓

ATTRACTANTS FIELD TEST

INTRODUCTION

One of the recommendations of the Screwworm Attractants Workshop held 4-5 October 1988 in Fargo, ND was to conduct a field test in Costa Rica to measure the effectiveness of swormlure (SL-4) in comparison with wounded animals, liver, and rehydrated blood. A meeting was held in San Jose, Costa Rica 16-17 February 1989 to discuss test rational and past attractants research.

Participants at the San Jose meeting were the following:

Team Members: D. Friese, ARS, Tuxtla Gutierrez

L. Hammack, ARS, Fargo

F. D. Parker, ARS, Costa Rica

R. D. Peterson II, ARS, Fargo

J. B. Welch, ARS, Costa Rica

What follows is a comparison of test methodology along with the advantages, disadvantages, and limitations of each. The test constraints are then discussed with general comments on feasibility in the Costa Rican context. This is followed by the team members recommendations and preliminary plans for a field test.

REVIEW OF METHODOLOGY

All the attractants have been tested in Costa Rica or have been studied previously from Texas to Guatemala. Generally, liver has caught the most screwworms, followed by wounds, wormlure and rehydrated blood (TABLES 1 & 2). The validity of comparisons among these studies is often compromised by methodological differences. The variances among rates of capture can be attributed to variation in attractant, screwworm population density, trap efficiency, climate, season, and/or suitability of trap sites.

A field test would compare relative attractiveness based on the number of flies captured while visiting each bait. The attractants could be tested concurrently using any of the following methods:

- A. Traditional. Using methods described in the literature.
- B. Standard. Using methods currently in use.
- C. Quantitative. Materials placed on the ground with uniform exposed surface area.

A. Traditional Method. Using methods described in the literature.

1. Infested animal wound. Egg masses collected twice daily.

Advantages:

- a. Wounded animals are used in strain evaluations.
- b. A reference point for comparison to past results.

Disadvantages:

- a. Egg mass numbers are not a valid indicator of attractiveness as not all flies visiting wounds oviposit.
- b. Wounded animals can only be used in restricted areas.
- c. Cost of animal transport and maintenance.

2. Rehydrated blood in a wind oriented trap (WOT) or Bishopp trap.

Advantage: None

Disadvantages:

- a. No field data on suitability for use in either trap.
- b. Unknown initial chemical composition or over time.
- c. Frequent theft of traps in all but selected areas.

3. Swormlure-4 used in a WOT.

Advantages:

- a. Field standard as used in strain evaluations, etc.
- b. Known initial chemical composition.
- c. A reference point for comparison with past results.

Disadvantages:

- a. Unknown whether Costa Rica government would approve SL-4 use in more than a small scale field test.
- b. Frequent theft of traps in all but selected areas.
- c. Handling of toxic material.

4. Liver used in a Bishopp Trap.

Advantage: Standard trapping system before the WOT.

Disadvantages:

- a. Traps large numbers on non-target species.
- b. Identification is labor intensive.
- c. Frequent theft of traps in all but selected areas.

The limitations of the Traditional Method are as follows:

1. No uniform context for presentation of attractant (WOT, animal, Bishopp).
2. Replications of the four presentations would be different, ie. could not have as many wounded animals as WOT traps.
3. Trap efficiency and type of attractant cues are different.
4. The spacing between replicates and treatments would require a large area.
5. A study area of sufficient size does not at present exist.

B. Standard Method. Using methods currently in use.

1. Infested animal wound. Flies collected on the bait by a technician.

Advantages: All screwworm visits to the bait are recorded by a technician.

Disadvantages:

- a. Wounded animals can only be used in restricted areas.
- b. Cost of animal transport and maintenance.
- c. Labor intensive.

2. Rehydrated blood on the ground. Flies collected on the bait by a technician.

Advantages: All screwworm visits to the bait are recorded by a technician.

Disadvantages:

- a. Periodic additions of rehydrated blood
- b. Labor intensive.

3. Swormlure-4 used in a WOT.

Advantages:

- a. Field standard as used in strain evaluations, etc.
- b. Known initial chemical composition.
- c. A reference point for comparison with past results.

Disadvantages:

- a. Unknown whether Costa Rica government would approve SL-4 use in more than a small scale field test.
- b. Frequent theft of WOTs in all but very selected areas.
- c. Handling of toxic material.
- d. Visiting flies must enter the WOT to be recorded.

4. Liver on the ground. Flies collected on the bait by a technician.

Advantages:

- a. All screwworm visits to the bait are recorded by a technician.
- b. Presentation as used in ongoing population studies.
- c. Inexpensive
- d. Nontoxic to technicians and the environment.

Disadvantages:

- a. Periodic turning of the liver and additions of water.
- b. Labor intensive.

The limitations of the Standard Method are as follows:

1. Replication would require more than doubling present manpower.
2. Presentation of attractant (WOT, animal, human, ground) is not uniform.
3. Replications of the four presentations would be different, ie. could not have as many wounded animals as WOT traps.
4. Trap efficiency and type of attractant cues are different.
5. The spacing between replicates and treatments would require a large area.
6. A study area of sufficient size does not at present exist.

C. Quantitative Method. Test material placed on the ground with uniform exposed surface area and an observer/recorder.

1. Wound fluid. Soaked on cotton in uniform sized container.

Advantages: More uniform context for attractant presentation.

Disadvantages:

- a. Quantity of wound fluid would be prohibitive.
- b. Collection, storage, and transport of fluid to the field.
- c. Maintenance of an animal source for the fluid.
- d. Periodic additions of wound fluid.
- e. Labor intensive.

2. Rehydrated blood. Soaked on cotton in uniform sized container.

Advantages: More uniform context for attractant presentation.

Disadvantages:

- a. Periodic additions of rehydrated blood.
- b. Labor intensive.

3. Swormlure-4. Soaked on cotton in uniform sized container.

Advantages:

- a. More uniform context for attractant presentation.
- b. Known chemical composition.

Disadvantages:

- a. Periodic additions of SL-4.
- b. Labor intensive.
- c. Unknown whether Costa Rican government would approve SL-4 use in more than a small scale field test.
- d. Handling of toxic material.

4. Liver. Cut to fit uniform sized container.

Advantages: More uniform context for attractant presentation.

Disadvantages:

- a. Periodic turning of the liver and additions of water.
- b. Labor intensive.
- c. All attractants but liver presented as liquids on cotton.

The limitations of the Quantitative Method are as follows:

1. Replication would require more than doubling present manpower.
2. The spacing between replicates and treatments would require a large area.
3. A study area of sufficient size does not at present exist.
4. Presentation efficiency for type of attractant is compromised.

GENERAL COMMENTS: The Costa Rican Context

The comparison of infested wounds, liver, rehydrated blood and SL-4 as outlined above presents problems of interpretation due to the inherent variability of the four attractants and the differences in their presentation. For example, some infested wounds attract more flies than others. This is also true for liver due to factors such as its state of decomposition. A comparison between the attractants as they are used in the field ie. SL-4 in a WOT, infested animal wounds, liver on the ground, incurs the effect of trap placement and efficiency in some treatments and not others.

Over the years, the screwworm staff in Costa Rica has determined in which areas field research can be productively carried out. Destruction of habitat and theft of traps and animals has limited the availability of study areas. As a deterrent to theft, it has been suggested that guards be hired to patrol trap lines and penned animals. However, even assuming the reliability and effectiveness of a guard, the cost (\$90/day) is prohibitive. There is the possibility (negotiations currently in progress) that limited studies can be conducted at the La Selva Biological Station (tropical wet habitat), however, the introduction of animals into the area is prohibited. There is no study area of sufficient size and security that is available in the premontane tropical habitat. Only one study area, the Ministry of Agriculture's experiment station, Enrique Jimenez Nunez, near Canas (tropical dry forest), has proved satisfactory for field studies (See MAP). The area is relatively small when compared to the area needed to test the four substances concurrently.

It was recommended that swormlure (SL-4) not be used as the population indicator to which the other attractants are compared. It was felt that a population indicator should be able to reliably attract screwworms in a variety of situations. Swormlure baited traps have generally caught fewer flies compared with liver or infested animals (TABLE 1 & 2). Swormlure is expensive, toxic, requires special handling, and it is unclear whether Costa Rican government approval is necessary before swormlure can be put into the environment. It was recommended that liver be used as the population indicator to which comparisons are made. The choice of liver is based on its low cost, ease of use, and its ability to attract substantial numbers of screwworms everywhere in Costa Rica where it has been tested (TABLE 2).

A large scale field test would require the hiring and training of additional technicians and further strain the resources of the Costa Rica laboratory. There are presently 4 technicians available for field work, and any large scale test would require at least doubling this figure. Bringing in trained technicians from Mexico would hinder Tuxtla's activities and test Costa Rican labor and immigration law.

RECOMMENDATIONS

There was mixed opinion among the San Jose participants as to the rational for conducting a field test. Based on scientific merit, however, there was agreement that a field test was not warranted. If after considering our evaluation of test methodology and past research the reviewers consider that a field test is warranted, we recommend that a paired comparison between liver and swormlure be conducted during the wet and dry season in two ecological zones of Costa Rica. We expect the liver to be an excellent indicator of screwworms in the study area. It is recommended that liver vs infested animals not be compared because of the lack of standardized presentation; and that liver vs rehydrated blood not be compared because the blood has been shown to be a poor attractant for screwworms in the field (TABLE 2).

It was proposed that the initial studies could be conducted at the experiment station near Canas (tropical dry habitat), and depending on the results, the study could be repeated in a second ecological zone, perhaps at the La Selva Biologicaal Station (tropical wet habitat). The restriction on using live animals in La Selva has been previously noted. Likewise, the importation of the swormlure ingredients and permission to use SL-4 in Costa Rica need to be worked out.

The paired comparison would run 10 consecutive days as this was considered sufficient time to give each attractant equal advantage in attracting different segments of the population ie. newly emerged, feeding, mating or ovipositing. The attractants will be presented in uniform sized containers set on the ground.

There would be two test sites for the paired comparison ie. one liver station and one SL-4 station. The treatments would be rotated daily to minimize any bias between sites. It will be necessary to make periodic additions of SL-4 to prevent the solutions from drying out. Additions of water and periodic turning of the liver will also be necessary to maintain its attractiveness. A technician will observe each treatment, capture the flies by net or vial upon visitation, hold those captured, and release them at the end of each day. This will minimize recaptures of released flies. Duration of the daily exposure will include the time when screwworms are known to be active.

Numbers of flies captured will be recorded by sex. The t-test for paired comparisons will be used to evaluate the treatments. The paired t-test is especially appropriate for this type of data to eliminate a source of extraneous variance (Steel and Torrie 1960).

REFERENCES

- Brenner, R. J. 1984. Dispersal, mating, and oviposition of the screwworm (Diptera: Calliphoridae) in southern Mexico. *Ann. Entomol. Soc. Amer.* 77:779-788.
- Brenner, R. J. 1985. Distribution of screwworms (Diptera: Calliphoridae) relative to land use and topography in the humid tropics of southern Mexico. *Ann. Entomol. Soc. Amer.* 78:433-439.
- Broce, A. B., J. L. Goodenough, and J. R. Coppedge. 1977. A wind oriented trap for screwworm flies. *J. Econ. Entomol.* 70(4):413-416.
- Coppedge, J. R. and J. W. Snow. 1977. Seasonal response of screwworm adults to two attractants in subtropical Texas. *Southwest Entomol.* 2(2):57-61.
- Coppedge, J. R., E. Ahrens, J. L. Goodenough, F. S. Guillot, and J. W. Snow. 1977. Field comparisons of liver and a new chemical mixture as attractants for the screwworm fly. *Environ. Entomol.* 6(1):66-68.
- Coppedge, J. R., J. L. Goodenough, A. B. Broce, F. H. Tannahill, J. W. Snow, M. M. Crystal, and H. D. V. Petersen. 1978. Evaluation of the screwworm adult suppression system (SWASS) on the island of Curacao. *J. Econ. Entomol.* 71(4):579-584.
- Jones, C. M., J. W. Snow, and M. A. Villasenor. 1976. Screwworm flies: Seasonal occurrence in central Tamaulipas, Mexico, 1973-74. *J. Econ. Entomol.* 69(6):761-762.
- Krafsur, E. S. and B. G. Hightower. 1979. Field tests of sterile screwworm flies, Cochliomyia hominivorax (Diptera: Calliphoridae), against natural populations in three coastal areas of Mexico. *J. Med. Entomol.* 16(1):33-42.
- Lindquist, A. W. 1938. A study of the incidence and habits of Cochliomyia americana by means of flytraps. *J. Kan. Entomol. Soc.* 11(3):97-104.
- Mackley, J. W. 1986. Incidence of the screwworm, Cochliomyia hominivorax, and the secondary screwworm, C. macellaria (Diptera: Calliphoridae), in the central highlands of Chiapas, Mexico. *J. Med. Entomol.* 23:76-82.
- Mackley, J. W. and H. E. Brown. 1984. Swormlure-4: A new formulation of swormlure-2 mixture as an attractant for adult screwworms, Cochliomyia hominivorax (Diptera: Calliphoridae). *J. Econ. Entomol.* 77:1264-1268.
- Spencer, J. P., C. J. Whitten, J. R. Coppedge, and J. W. Snow. 1980. Comparison of screwworm captures in liver- and swormlure-baited traps in a tropical area of southern Mexico. *Southwestern Entomol.* 5(3):175-178.

Spencer, J. P., J. W. Snow, J. R. Coppedge and C. J. Whitten. 1981. Seasonal occurrence of the primary and secondary screwworm (Diptera: Calliphoridae) in the Pacific coastal area of Chiapas, Mexico during 1978-1979. J. Med. Entomol. 18:240-243.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill: New York. 481p.

Thomas, D. B. and R. L. Mangan. Oviposition and wound visiting behavior of the screwworm fly, Cochliomyia hominivorax (Coquerel)(Diptera: Calliphoridae). Ann. Entomol. Soc. Amer. (in press).

TABLE 1. RESPONSE OF WILD SCREWORM FLIES TO ANIMAL WOUND, LIVER, OR SWORMLURE (SL). DATA ARE EXPRESSED AS FLIES/STATION/DAY, WHERE A STATION REPRESENTS ONE TRAP (*WOT, **BISHOPP), AN OBSERVED ANIMAL WOUND, OR ONE LIVER-OBSERVATION SITE.

FLIES/STATION/DAY			AREA	DATE	REFERENCE
WOUND	LIVER	SL - ()			
	5.79**	-	Texas	7/15/35-10/30/37	Linguist 1938
2.8	9.60**	-	Texas	9/35 (10 days)	
6.5	14.9 **	-	Texas	7/36 (15 days)	
-	0.60**	-	Tamaulipas, MX	4/19/73-4/15/74	Jones et al. 1976
-	-	1.81* (2) 0.71** (2)	Texas	7/9-8/13/76	Broce 1977 (females only)
-	0.1 **	0.2 **	Texas	10/31/74-11/1/75	Coppedge & Snow 1977
-	4.50**	6.40** (2)	Texas	1975 (10 days)	Coppedge et al. 1977
-	-	0.22-1.12* (2)	Curacao	12/1/76-1/21/77	Coppedge et al. 1978
-	0.32**	-	Veracruz, MX	5/74-6/74	Krafsur & Hightower 1979
-	0.07**	0.08* (2)	Chiapas, MX	7/78-9/78	Spencer et al. 1980
-	-	0.12* (2)	Arriaga, MX	3/78-3/79	Spencer et al. 1981
-	-	0.12* (4)	Tapachula, MX	7/27/-11/25/82	Brenner 1984
-	-	0.13* (4)	Arriaga-Tonala, MX	11/17-11/25/82	Mackley & Brown 1984
-	-	0.03* (4)	Tapachula, MX	5/83-6/83	Brenner 1985
-	-	0.01* (2)	Chiapas, MX	1/13/80-1/15/81	Mackley 1986
-	-	0.01* (4)	Pital, CR	11/19-12/8/86	Welch (unpubl.)
-	-	0.13* (4)	Canas, CR	11/19-12/4/86	
1.31	-	-	Chetumal, MX	10/85-11/85	Thomas & Mangan
1.23	-	-	Orange Walk, BZ	8/86-9/86	(in press)
1.5	-	-	Guatemala	Apr.-May 87	Thomas et al. (unpubl.)
4.66	-	-	Pital, CR	10/31-11/15/86	Rubink & Ramirez
2.02	-	-	Canas, CR	9/30-12/14/86	(unpubl.)
0.92	-	-		3/20-5/20/87	

TABLE 2. RESPONSE OF WILD SCREWORM FLIES TO ANIMAL WOUND, LIVER, FISH, OR REHYDRATED BLOOD. DATA ARE EXPRESSED AS FLIES/STATION/DAY, WHERE A STATION REPRESENTS ONE TRAP (**BISHOPP), AN OBSERVED ANIMAL WOUND, ONE LIVER-OBSERVATION SITE, OR ONE REHYDRATED BLOOD-OBSERVATION SITE. DATA ARE FROM PARKER AND WELCH (UNPUBL.)

WOUND	FLIES/STATION/DAY			AREA	DATE
	LIVER	FISH	BLOOD		
3.0	-	-	-	El Alto, CR	1/29-2/19/88
2.8	-	-	-	Guapiles, CR	2/23-3/22/88
6.8	-	-	-	Canas, CR	3/9-3/22/88
2.8	6.2**	2.0**	-	Canas, CR	8/2-8/22/88
1.4	6.5	-	-	Pital, CR	9/12-9/23/88
-	5.8**	-	-		
-	12.7	-	-	Canas, CR	1/24-3/16/89
-	6.1	-	-	Canas, CR	3/17-3/24/89
-	3.1**	-	-		
-	15.0	-	0.0	Canas, CR	3/10-3/16/89





United States
Department of
Agriculture

Agricultural
Research
Service

Southern Plains Area

Screwworm Research Laboratory
Tuxtla Gutierrez, Chiapas, Mexico

3/21/91

10:55 am

FAX Number:
Within Mexico (961)-3-07-67
International 52-(961)-3-07-67

FAX TRANSMISSION

Status
of Workshop
Objectives

TO:

Ralph Bream

FROM:

Jim Whitten

SUBJECT:

Attractants Research, etc

DATE:

Mar 21 1991

REMARKS:

PAGES SENT INCLUDING THIS COVER SHEET:

7

IF THIS TRANSMISSION WAS NOT RECEIVED IN ITS ENTIRETY PLEASE NOTIFY
US IMMEDIATELY. TELEPHONE #(961)-3-35-50 (Within Mexico)
52-(961)-3-35-50 (International)

March 20, 1991

Ralph, Recommendations for Location of Screwworm Research in the U.S.

Status of Attractants Research

As per your request, Frank and Dan have compiled a status report of the attractants research priorities as developed at the workshop in 1988. I am sending by this FAX the reports by Frank and Dan. The report from Frank may not be very legible as it is a copy of a FAX we received from him. If you have any questions, I will be in the lab on Friday, March 22.

Both reports mention the need for alternate bioassay techniques. The workshop report suggested the use of a wind tunnel. Another technique is the use of electroantennograms (EAG). The British research group is using EAG technology to screen response of screwworm adults to various chemicals. Apparently this system is very sensitive and does not harm the fly. The problem with this technique is that when a response is indicated, one does not know whether the response is one which will affect the behavior of the insect - ie. attraction, repellency, or what. Perhaps with initial screenings using EAG to identify potential chemicals and a follow-up bioassay in an olfactometer or wind tunnel, one could better determine the behavioral response of the flies.

The need for a dedicated chemist is obvious if we are to increase our knowledge of attractancy and wound ecology. This chemist should be assigned to work primarily (or only) with the research objective at hand. And the nearer the chemist is located to the bioassay laboratory, the greater the possibility of repeatable results.

I too believe that there is no need to attempt to further improve the formulation of Swormlure. There are some systems available that we can test to make the deployment of the attractant more useful in terms of longevity and safety.

The ideal attractant situation would be to understand enough about wound ecology to be able to develop an artificial wound. All previous work has been oriented to making an artificial wound by mimicing the wound (or larval habitat) itself. We simply do not know enough about the wound in specific characteristics to have a high probability of success.

We have learned much about the field behavior of flies around a wound or an attractant such as liver. The problem still remains as to what is controlling that behavior.

Testing candidate materials in the field can readily be accomplished at both Mexico and Costa Rica. Both laboratories will be receptive to any suggestions/recommendations.

page 2

Considerations for Location of Screwworm Research in the U.S.

Fargo was considered to be an ideal location for conducting research with the screwworm in the U.S. both in terms of the climatological and research environments. Research in the areas of screwworm genetics and attractants were primary objectives.

In view of proven potential, I feel that the genetics research should be given priority. Dave Taylor has completed much of the preliminary work necessary to really investigate the formal genetics of the screwworm. While the work could be completed at another location, time would be lost in establishing a laboratory. Also, in the past, cooperation with other insect geneticists at Fargo has proven to be beneficial for the screwworm mission with a minimum of expense. I do not know if this situation still exists. Dave is in a much better position to evaluate this potential. Cooperative research not requiring live insects could still be accomplished at Fargo if the secured rearing lab were at another location.

The attractants research has been ongoing since 1976 and some progress has been made. However, at the present the potential for further progress appears to be less than that of genetics research. This situation could perhaps be corrected with reorientation of research objectives, methodology, and/or personnel. Attractants research is not easy but yet has great potential for use in the eradication program. Some of the problem may be attributed to a lack of perspective of the needs of the eradication program and of livestock insects research in general.

Interest is usually greater when one feels that he/she is an integral part of the total program. In Fargo, screwworm research is a small cog in a big wheel that is not closely associated with the goal of screwworm eradication.

Results for Objective 3.

1. Test effectiveness of swormlure. Swormlure was not available to test in Costa Rica until just recently. We are planning a small field test in the dry season in Canas in April and later in the wet forest at Bijagua because I am curious about the effectiveness of the WOT trap. I would like to observe fly activity at the attractant source. It was the opinion of the ad hoc committee, however, that results of previous studies suggest swormlure was not effective in tropical habitats and therefore, further tests were not warranted. Numbers of flies attracted per trap per station per week were exceedingly few when baited with swormlure (range 0.2 - 0.7). Results with liver as an attractant have been better in Costa Rica and in Belize (range 24 - 161).

Dried blood was field tested by myself and by Leslie when she was here in 1989. No flies were attracted including the many other species and genera of common blowflies in the area. During the same period and at the same site where the blood was tested, rotted liver was set out and screwworm adults were attracted immediately. On some days as many as 50 females were collected at one site using liver, but none were attracted to the dried blood mixture. These results raise interesting questions concerning all the work with olfactometers and dried blood as the standard, especially when rotted liver was tested in the olfactometer and no or very few responses from screwworms were observed. Why is liver such as reliable attractant in the field and of little value in the laboratory? Either the olfactometer is inadequate for such tests or the reared strains have lost their abilities to respond. I hope it is not the latter.

A direct comparison of sentinel animals (wounded sheep) and rotted liver was done in Costa Rica in 1989 and 1990. These studies were done in both the wet and dry seasons and in 3 habitats. Results were highly skewed towards liver as the best attractant and to woods as the preferred habitat. For example, in the woods liver attracted 4.1 times more flies (1540 vs 378) than did sentinel animals and at the edge of the woods liver attracted 4.8 times more (1899 vs 395). Screwworm adults infrequently visited pastures, contrary to much of the existing literature, and less than 77 flies were attracted to either source. More flies (1.4 to 2.4 times) visited sheep in the wet than in the dry season, except in the deep woods where 0.9 times more were recorded during the dry season. More adults were attracted to liver in the wet season (2.7 to 3.2 times) than the dry. Sentinel animals appear to attract only a small portion of the actual screwworm population in an area and in my opinion that the flies are responding to other attractants in the area (wild animals). Our initial results with the ELISA host feeding tests support these conclusions. Only 5% of the flies had fed on livestock (beef) but 33% had evidence of feeding at our test sheep in the woods. More importantly, 62% of the flies had fed on other but unidentified animals. These results suggest that wild animals are the primary source for screwworm population in our area and livestock is not very important to the population dynamics of this pest species. This is not to say that the screwworm is not an economic problem here, but the pest problem is acerbity more by wild animals than by livestock.

2. Define host related phenomena. Nothing to report.

3. Wound ecology. Preliminary tests have been done with swabs contaminated with wound fluids. Such swabs placed in shrubs attracted female screwworms that were gravid. Development of such an attractant is needed because less than 1% of 726 females were gravid that had been collected at liver baits and dissected.

4. Improvement of Swormlure. No data to make this decision.

5. Artificial Wounds. Some models have been tested that used hot water as the heat source. No eggs were obtained in the field, but caged flies did oviposit in one test. A deer model is being used for further tests.

6. Plant Attractants. Pollen identification has been the major problem in obtaining results for this objective. Currently a person in Columbia (Bogota) is doing the identifications. When these are returned we can determine the degree of priority for this objective. Preliminary observations suggest that the flies have a narrow range of plants visited for nectar at a given location. Obviously nectar is vital in their diet as nearly all flies have evidence of feeding, especially during their early ovarian stages. Males are heavily contaminated with pollen and those dissected are usually gorged with nectar. After oviposition, and especially when a host is repeatedly visited, plant nutrients may be less depended upon. Identified host plants may be an important means of determining favorable habitats using remote sensing techniques.

Floral visitation by sterile and fertile *Cochliomyia vicina* were conducted in Guatemala. The results of these studies suggested that floral visitation by both sterile and fertile adults were identical and more importantly, that plant visitation patterns had not been altered by laboratory adaptation.

To: R.A. Bram

From: D.D. Friese - Screwworm Research Lab - Tuxtla Gutierrez

Subject: Status of attractants research with regard to
priorities set at 1988 ARS Workshop on Screwworm
Attractants.

Date: 18-March-1991

As you are aware at the conclusion of the ARS Screwworm Attractants Workshop held in Fargo, ND in October 1988 participants discussed future attractants research and attempted to place research emphasis and priorities on future research. The results of this discussion were summarized in a report sent out by Dr. Whitten in November 1988.

In response to your request for my input I will confine my comments to sections B and C found on pages 3 and 4 of Dr. Whitten's report. The first priority for future attractants research noted by Dr. Whitten (B.1.) was to conduct a field comparison of known and potential attractants to assess the effectiveness of each. As a direct result of this being assigned top priority a research meeting was held in San Jose, Costa Rica in February of 1989. Participants included myself, Drs. Hammack and Peterson from the Fargo lab and Drs. Parker and Welch from the Costa Rica lab. Topics discussed at this meeting were the rationale for a field test, a comparison of the test methodology including advantages and disadvantages of various test designs and the feasibility of conducting such a test in Costa Rica. The results of these discussions and the recommendations of the team members were summarized by Dr. R.D. Peterson in a memo of April 30, 1989. Although a design for a field test was included, as per our recommendations a field test was considered unwarranted.

The second recommendation of Dr. Whitten's summary was a long term research emphasis defining host related attractant phenomena (B.2.). Progress in this area has been made both in the Fargo and Tuxtla locations (B.2.a.1-2.). At present a reliable method of bioassay (B.2.a.1.) exists in the Fargo lab in the form of a multiport olfactometer. Comparative trials are possible using a dried blood source as standard. In regard to sampling and collecting potential volatiles (B.2.a.2.), a wound fluid collector has been designed and constructed by myself and my technician and several models have been tested at the Tuxtla location. The final model allows for efficient collection of fluid and ease in freezing the samples collected in liquid nitrogen for storage or transport. Recent cooperative work involving the collection of wound fluid samples at the Tuxtla location and their transport to Fargo for testing in the olfactometer was successful. Wound fluid was collected daily during three four hour intervals from four animals infested with irradiated screwworm larvae. Fluid was frozen

immediately after collection and maintained frozen. Samples were transported in liquid nitrogen to Fargo where they underwent testing in the olfactometer. Results of the test with mated, gravid screwworm females indicated no attractiveness of wound fluid from 68-92 hours post infestation. From 92-103 hours post infestation wound fluid showed attractiveness similar to that of the dried blood standard.

As far as I am aware very little progress has been made on the last three points under attraction (B.2.a.3-5.). At present the screwworm group in Fargo has the support of a chemist, but I think this support is much less than full time and ancillary to his primary responsibilities. In conversations with Dr. Hammack we are both in agreement that, until the support of a full time chemist who is both interested and committed to this project occurs, little progress will likely be made in these areas.

Although we have made progress in the area of bioassay and attractants collection with the methods currently at our disposal I am also aware that other technologies exist which may be utilized. One very beneficial outcome of the visit of Drs. Hall and Torr to Tuxtla has been my ability to view first hand the collection methods used by them to collect volatiles from the wound. They were able to collect volatiles using a vacuum pump connected to a chamber which had an inlet which passed carbon filtered air over the wound and exited this air thorough a column which trapped the volatiles. This type of device could be used here in Tuxtla to collect volatiles from wounded and infested animals. I was also very interested in the bioassay methods which their group in England had used in many of the tsetse fly studies. Apparently they have relied quite heavily on an electroantennagram which is quite sensitive to attractants. Again this methodology could possibly be adapted to our work with the screwworm. In addition it was related to me by Dr. Torr that their group in England is very keen in working on projects such as ours and there exists the possibility of future collaboration with this group if the technical difficulties could be worked out. Samples of volatiles, wound fluid, waste diet and the oviposition attractant we use in the lab were supplied to Dr. Torr for use by his group.

The only progress made here in Tuxtla on the other points listed in Dr. Whitten's report has been in the area of host physiology (B.3.c.). I have made a fairly extensive literature search on wound healing and the immune response. The wound healing response and the physiological processes in humans and other mammals is quite well known. A fairly detailed sequence of events can be constructed for surgically sterile wound healing and the various mediators have been identified as to the sequence of their presence in the healing wound. What is not well known and what is pertinent to our concerns is how the healing process is affected by a screwworm infestation. I think that this could lead to a very interesting study which could possibly identify a host of chemicals which are in the wound concurrent with screwworm larvae and which could have important activity both from an attractant as well as a nutritional point of view. Although it would be possible to do the animal work here in Tuxtla, as noted above it would require the participation of a chemist to make any reasonable progress.

